

Listing of Claims:

1. (Currently Amended) A fluorescence energy transfer dye comprising Formula I:

--L1—D1—FETL—D2—L2— (Formula I)

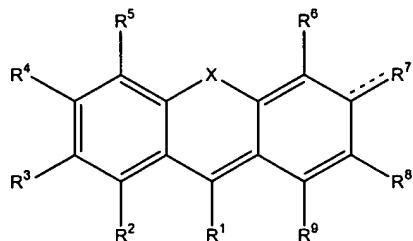
where:

L1 is a link for attachment to a probe or target, for attachment to a solid support, or is absent;

L2 is a link for attachment to a probe or target, for attachment to a solid support, or is absent;

FETL is a fluorescence energy transfer linker; comprising a symmetric, rigid or sterically hindered, divalent moiety joined to D1 and D2 via an amine, carbonyl, activated carboxylic acid ester, disulfide, thiol or thiol ester;

D1 is a donor dye represented by the formula:



D1

where:

X is O or C(R^{*}R^{**}), where R^{*} and R^{**} are independently lower alkyl or —CH₂—Z;

R¹ is H, CF₃, perfluoropropyl, lower alkyl acid, substituted aryl, substituted heteroaryl or Z;

R² is H, halo, SO₃[−], or is taken together with R³ to form an optionally substituted fused ring having 5 to 7 atoms;

R³ is halo, Z, or is taken together with R² and/or R⁴ to form an optionally substituted fused ring having 5 to 7 atoms;

R⁴ is =O or OH, -N(R⁴R⁴′) or =N⁺(R⁴R⁴′), or is taken together with R³ and/or R⁵ to form an optionally substituted fused ring having 5 to 7 atoms,

where R⁴ is H, lower alkyl or L1, and

R⁴′ is H, lower alkyl or CH₂—Z;

R⁵ is H, halo, Z, or is taken together with R⁴ to form an optionally substituted fused ring having 5 to 7 atoms;

R⁶ is H, halo, Z, or is taken together with R⁷ to form an optionally substituted fused ring having 5 to 7 atoms;

R⁷ is =O or OH, -N(R⁷R⁷′) or =N⁺(R⁷R⁷′), or is taken together with R⁶ and/or R⁸ to form an optionally substituted fused ring having 5 to 7 atoms,

where R^7 is H, lower alkyl or L1, and

R^7 is H, lower alkyl or CH_2-Z ;

R^8 is halo, Z, or is taken together with R^7 and/or R^9 to form an optionally substituted fused ring having 5 to 7 atoms;

R^9 is H, halo, SO_3^- , or is taken together with R^8 to form an optionally substituted fused ring having 5 to 7 atoms; and

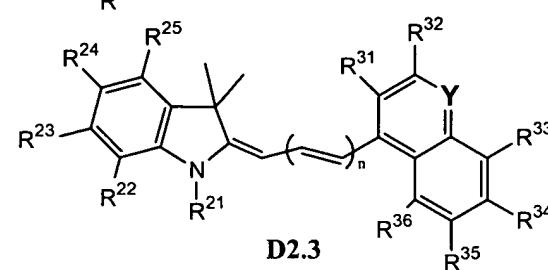
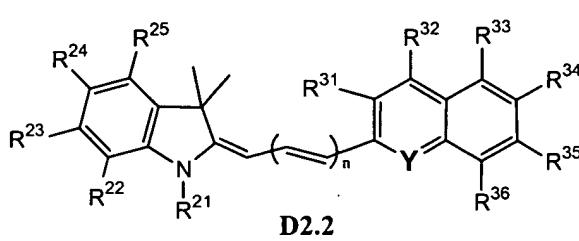
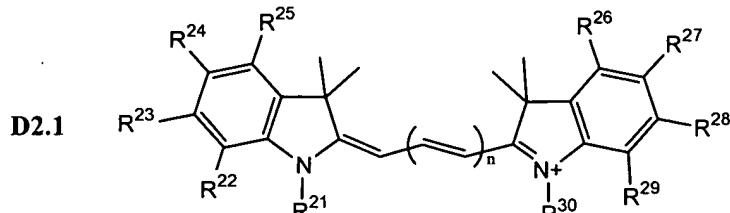
Z is a group of the formula: $-Z^*-Z^1-$ (linkage to L1, L2 or FETL), where:

Z^* is methylene, methoxy, ethoxy, aminomethyl, aminoethyl, aminopropynyl, aminobutynyl, carboxyethenyl, carboxyethynyl, optionally substituted aryl or optionally substituted heteroaryl;

Z^1 is $-C(O)-$, $-N(Z^2)-$, $-CH_2-O-$, $-CH_2-C(O)-$, $-CH_2-N(Z^2)-$, $-CH_2-S-$, $-CH_2-S(O)-$, $-CH_2-S(O_2)-$ or is absent; and

Z^2 is H, C_1 to C_8 optionally substituted lower alkyl, or optionally substituted aryl; and

D2 is an acceptor/reporter dye represented by formula D1 or by a formula of the group:



where:

at least one of R^{21} to R^{36} is joined to FETL,

n is zero, 1, 2 or 3;

R^{21} and R^{30} are independently $-CH_2-Z$, activated lower alkyl, or optionally substituted aryl;

R^{22} to R^{29} are independently H, SO_3^- , or optionally substituted alkyl, or R^{22} and R^{23} , R^{23} and R^{24} , R^{24} and R^{25} , R^{26} and R^{27} , R^{27} and R^{28} , and/or R^{28} and R^{29} taken together form an optionally substituted fused ring having 6 atoms;

R^{31} and R^{32} are independently H, optionally substituted alkyl, aryl, or taken together form an optionally substituted fused ring having 6 atoms;

R^{33} to R^{36} are independently H, SO_3^- , optionally substituted alkyl, aryl, or R^{33} and R^{34} , R^{34} and R^{35} , and/or R^{35} and R^{36} taken together form an optionally substituted fused ring having 6 atoms; and

Y is $-O-$ or $-N(Y^1)-$ where Y^1 is $-CH_2-Z$, activated lower alkyl, or optionally substituted aryl;

provided that at least one of R^3 to R^8 is Z where Z^* is optionally substituted aryl or optionally substituted heteroaryl, or a probe-, target- and/or support-conjugate thereof.

2. (Currently Amended) The fluorescence energy transfer dye of Claim 1 having one or more of the following:

R^1 is H, CF_3 , perfluoropropyl, lower alkyl acid, 5-6 membered mono or 10-12 membered fused substituted aryl or heteroaryl, or Z;

R^2 is H, halo, SO_3^- , or is taken together with R^3 to form an optionally substituted fused 6-membered aryl ring;

R^3 is halo, Z, or is taken together with R^2 and/or R^4 to form an optionally substituted fused 6-membered ring;

R^4 is $=O$ or OH, $-N(R^4R^4)$ or $=N^+(R^4R^4)$, or is taken together with R^3 and/or R^5 to form an optionally substituted fused 6-membered ring;

R^5 is H, halo, Z, or is taken together with R^4 to form an optionally substituted fused 6-membered ring;

R^6 is H, halo, Z, or is taken together with R^7 to form an optionally substituted fused 6-membered ring;

R^7 is $=O$ or OH, $-N(R^7R^7)$ or $=N^+(R^7R^7)$, or is taken together with R^6 and/or R^8 to form an optionally substituted fused 6-membered ring;

R^8 is halo, Z, or is taken together with R^7 and/or R^9 to form an optionally substituted fused 6-membered ring;

R^9 is H, halo, SO_3^- , or is taken together with R^8 to form an optionally substituted fused 6-membered aryl ring;

R^4 is $-N(R^4R^4)$ or $=N^+(R^4R^4)$ and R^7 is $-N(R^7R^7)$ or $=N^+(R^7R^7)$ when X is $C(R^*R^{**})$;

Z is a group of the formula: $-Z^*-Z^1-$ (linkage to L1, L2 or FETL), where:

Z^* is methylene, methoxy, ethoxy, aminomethyl, aminoethyl, aminopropynyl, aminobutynyl, carboxyethenyl, carboxyethynyl, optionally substituted aryl or optionally substituted heteroaryl,

Z^1 is $-C(O)-$, $-N(Z^2)-$, $-CH_2-O-$, $-CH_2-C(O)-$, $-CH_2-N(Z^2)-$, $-CH_2-S-$, $-CH_2-S(O)-$, $-CH_2-S(O_2)-$ or is absent, and

Z^2 is H, or is C_1 to C_8 lower alkyl or aryl optionally substituted with SO_3^- , $COOH$, NH_2 , CH_2NH_2 , SH , or SCH_3 ;

FETL comprises a symmetric, rigid or sterically hindered, divalent moiety joined to D1 and D2 via an amine, carbonyl, activated carboxylic acid ester, disulfide, thiol or thiol ester;

R^{21} or R^{30} is carboxy-naphthyl-methyl, mono- or di-*ortho*-substituted benzyl having an *ortho*-, *meta*- or *para*- carbonyl or activating group, or an activated C_1 to C_6 lower alkyl;

R^{22} to R^{29} are independently H, SO_3^- or optionally substituted alkyl, or R^{22} and R^{23} , R^{23} and R^{24} , R^{24} and R^{25} , R^{26} and R^{27} , R^{27} and R^{28} , and/or R^{28} and R^{29} taken together form a fused, sulfonated 6-membered aryl ring;

R^{31} and R^{32} are independently H, optionally substituted alkyl, aryl, or taken together form a fused, sulfonated 6-membered carbocyclic or heterocyclic ring;

R^{33} to R^{36} are independently H, SO_3^- , optionally substituted alkyl, aryl, or R^{33} and R^{34} , R^{34} and R^{35} , and/or R^{35} and R^{36} taken together form a fused, sulfonated 6-membered carbocyclic or heterocyclic ring; and/or at least one of R^{22} to R^{29} or R^{33} to R^{36} is SO_3^- .

3. (Currently Amended) The fluorescence energy transfer dye of Claim 2 having one or more of the following:

R^1 is H, CF_3 , perfluoropropyl, lower alkyl acid, an optionally substituted *ortho*-benzoic acid, or Z ;

R^2 is H, halo or SO_3^- ;

R^3 is halo or Z ;

R^4 is $=O$ or OH, $-N(R^4R^4)$ or $=N^+(R^4R^4)$;

R^5 is H, halo or Z ;

R^6 is H, halo or Z ;

R^7 is $=O$ or OH, $-N(R^7R^7)$ or $=N^+(R^7R^7)$;

R^8 is Z ;

R^9 is H, halo or SO_3^- ;

Z is a group of the formula: $-Z^*Z^1$ - (linkage to L1, L2 or FETL), where:

Z^* is an optionally substituted aryl or heteroaryl of the group: phenyl, adamantly, norboranyl, biphenyl, naphthyl, furanyl, bifuranyl, thiophenyl, bithienyl, pyrrolyl, indolyl, isoindolyl, benzofuranyl, isobenzofuranyl, benzothiophenyl, benzothienyl, chromenyl and isochromenyl, and

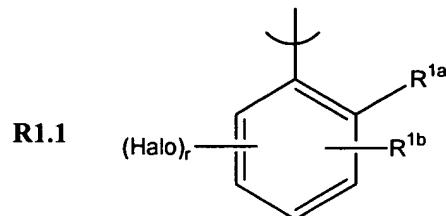
Z^1 is $-C(O)-$, $-N(H)-$, $-CH_2-O-$, $-CH_2-C(O)-$, $-CH_2-N(H)-$, or is absent;

R^{22} to R^{29} are independently H, SO_3^- or optionally substituted alkyl, or R^{24} and R^{25} and/or R^{26} and R^{27} taken together form a fused, sulfonated 6-membered aryl ring;

R^{31} and R^{32} are independently H, optionally substituted alkyl or aryl; and/or

R^{33} to R^{36} are independently H, SO_3^- , optionally substituted alkyl or aryl.

4. (Currently Amended) The fluorescence energy transfer dye of Claim 3 where R^1 is a group represented by Formula R1.1:



where:

R^{1a} is H, halo or $-C(O)O^-$;

R^{1b} is H, halo or $-C(O)-$ (linkage to L1, L2 or FETL);

R^{1c} is halo; and

r is 0, 1, 2 or 3.

5. (Original) The fluorescence energy transfer dye of Claim 1 where at least one of R^3 to R^8 is Z where Z^* is optionally substituted aryl selected from phenyl, adamantly, norboranyl, biphenyl and naphthyl.

6. (Original) The fluorescence energy transfer dye of Claim 5 where Z^* is phenyl and Z^1 is $-C(O)-$.

7. (Currently Amended) The fluorescence energy transfer dye of Claim 1 where:
 R^1 is H, CF_3 , perfluoropropyl, lower alkyl acid, an optionally substituted *ortho*-benzoic acid, or Z;

R^2 is H, halo or SO_3^- ;

R^3 is halo or Z;

R^4 is $=O$ or OH, $-N(R^4R^{4''})$ or $=N^+(R^4R^{4''})$;

R^5 is H, halo or Z;

R^6 is H, halo or Z;

R^7 is $=O$ or OH, $-N(R^7R^{7''})$ or $=N^+(R^7R^{7''})$;

R^8 is Z;

R^9 is H, halo or SO_3^- ;

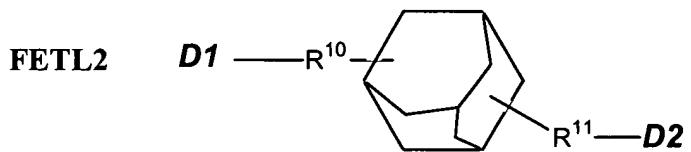
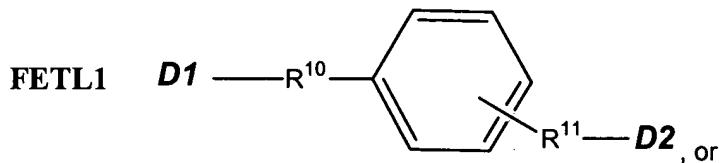
Z is a group of the formula: $-Z^*-Z^1-$ (linkage to L1, L2 or FETL), where:

Z^* is an optionally substituted aryl or heteroaryl of the group: phenyl, adamantly, norboranyl, biphenyl, naphthyl, furanyl, bifuranyl, thiophenyl, bithienyl, pyrrolyl, indolyl, isoindolyl, benzofuranyl, isobenzofuranyl, benzothiophenyl, benzothienyl, chromenyl and isochromenyl, and

Z^1 is $-C(O)-$, $-N(H)-$, $-CH_2-O-$, $-CH_2-C(O)-$, $-CH_2-N(H)-$, or is absent;

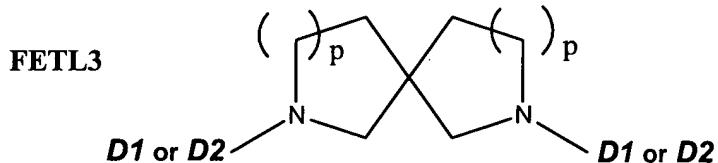
R^{21} or R^{30} is carboxy-naphthyl-methyl, mono- or di-*ortho*-substituted benzyl having an *ortho*-, *meta*- or *para*- carbonyl or activating group, or an activated C₁ to C₆ lower alkyl; R^{22} to R^{29} are independently H, SO₃⁻ or optionally substituted alkyl, or R^{24} and R^{25} and/or R^{26} and R^{27} taken together form a fused, sulfonated 6-membered aryl ring; R^{31} and R^{32} are independently H, optionally substituted alkyl or aryl; and R^{33} to R^{36} are independently H, SO₃⁻, optionally substituted alkyl or aryl.

8. (Currently Amended) The fluorescence energy transfer dye of Claim 1 where FETL is represented by a formula of the group:



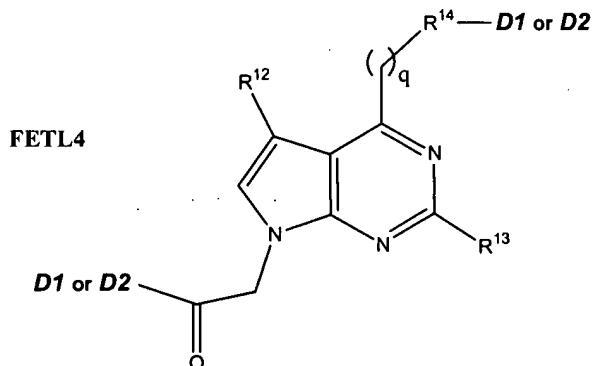
where:

R^{10} is $D1-C(O)-$, $D1-N(H)-CH_2-$, $D1-S-C(O)-$, $D1-O-C(S)-$ or $D1-S-CH_2-$; and
 R^{11} is $-C(O)-D2$, $-CH_2-N(H)-D2$; $-C(O)-S-D2$, $-C(S)-O-D2$ or CH_2-S-D2 ;



where:

p is independently 0, 1, 2 or 3; and



where:

q is zero or 1;

R^{12} is H, halo, or optionally substituted-alkyl, -alkenyl, -alkynyl or -aryl;

R^{13} is H, SO_3^- or optionally substituted-alkyl, -alkenyl, -alkynyl or -aryl; and

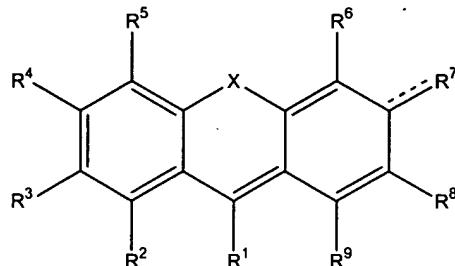
R^{14} is a secondary or tertiary amine or heterocyclil, particularly N(H) or piperazine.

9. (Currently Amended) The fluorescence energy transfer dye of Claim 8 where FETL is FETL1 and:

R^{10} is $D1-C(O)-$ or $D1-N(H)-CH_2-$; and

R^{11} is $-C(O)-D2$ or $-CH_2-N(H)-D2$.

10. (Currently Amended) An A compound represented by the formula:



D1

where:

X is O or $C(R^*R^{**})$, where R^* and R^{**} are independently lower alkyl or $-CH_2-Z$;

R^1 is H, CF_3 , perfluoropropyl, lower alkyl acid, substituted aryl, substituted heteroaryl or Z;

R^2 is H, halo, SO_3^- , or is taken together with R^3 to form an optionally substituted fused ring having 5 to 7 atoms;

R^3 is halo, Z, or is taken together with R^2 and/or R^4 to form an optionally substituted fused ring having 5 to 7 atoms;

R^4 is $=O$ or OH, $-N(R^4R^{4'})$ or $=N^+(R^4R^{4'})$, or is taken together with R^3 and/or R^5 to form an optionally substituted fused ring having 5 to 7 atoms,

where R^4 is H, lower alkyl or L1, and

$R^{4'}$ is H, lower alkyl or CH_2-Z ;

R^5 is H, halo, Z, or is taken together with R^4 to form an optionally substituted fused ring having 5 to 7 atoms;

R^6 is H, halo, Z, or is taken together with R^7 to form an optionally substituted fused ring having 5 to 7 atoms;

R^7 is $=O$ or OH, $-N(R^7R^{7'})$ or $=N^+(R^7R^{7'})$, or is taken together with R^6 and/or R^8 to form an optionally substituted fused ring having 5 to 7 atoms,

where R^7 is H, lower alkyl or L1, and

$R^{7'}$ is H, lower alkyl or CH_2-Z ;

R^8 is halo, Z, or is taken together with R^7 and/or R^9 to form an optionally substituted fused ring having 5 to 7 atoms;

R^9 is H, halo, SO_3^- , or is taken together with R^8 to form an optionally substituted fused ring having 5 to 7 atoms; and

Z is a group of the formula: $-Z^*-Z^1-$ (linkage to L1, L2 or FETL), where:

Z^* is methylene, methoxy, ethoxy, aminomethyl, aminoethyl, aminopropynyl, aminobutynyl, carboxyethenyl, carboxyethynyl, optionally substituted aryl or optionally substituted heteroaryl;

Z^1 is $-C(O)-$, $-N(Z^2)-$, $-CH_2-O-$, $-CH_2-C(O)-$, $-CH_2-N(Z^2)-$, $-CH_2-S-$, $-CH_2-S(O)-$, $-CH_2-S(O_2)-$ or is absent; and

Z^2 is H, C₁ to C₈ optionally substituted lower alkyl, or optionally substituted aryl;

L1 being a link for attachment to a probe or target, for attachment to a solid support, or is absent;

L2 being a link for attachment to a probe or target, for attachment to a solid support, or is absent; and

FETL being a fluorescence energy transfer linker comprising a symmetric, rigid or sterically hindered, divalent moiety joined to D1 and D2 via an amine, carbonyl, activated carboxylic acid ester, disulfide, thiol or thiol ester;

provided that at least one of R^3 to R^8 is Z, where Z^* is optionally substituted aryl or optionally substituted heteroaryl, or a FETL-, probe-, target- and/or support-conjugate thereof.

11. (Currently Amended) The compound of Claim 10 having one or more of the following:

R^1 is H, CF_3 , perfluoropropyl, lower alkyl acid, 5-6 membered mono or 10-12 membered fused substituted aryl or heteroafyl, or Z;

R^2 is H, halo, SO_3^- , or is taken together with R^3 to form an optionally substituted fused 6-membered aryl ring;

R^3 is halo, Z, or is taken together with R^2 and/or R^4 to form an optionally substituted fused 6-membered ring;

R^4 is $=O$ or OH, $-N(R^4R^4")$ or $=N^+(R^4R^4")$, or is taken together with R^3 and/or R^5 to form an optionally substituted fused 6-membered ring;

R^5 is H, halo, Z, or is taken together with R^4 to form an optionally substituted fused 6-membered ring;

R^6 is H, halo, Z, or is taken together with R^7 to form an optionally substituted fused 6-membered ring;

R^7 is $=O$ or OH, $-N(R^7R^7")$ or $=N^+(R^7R^7")$, or is taken together with R^6 and/or R^8 to form an optionally substituted fused 6-membered ring;

R^8 is halo, Z, or is taken together with R^7 and/or R^9 to form an optionally substituted fused 6-membered ring;

R^9 is H, halo, SO_3^- , or is taken together with R^8 to form an optionally substituted fused 6-membered aryl ring;

R^4 is $-N(R^4R^4)$ or $=N^+(R^4R^4)$ and R^7 is $-N(R^7R^7)$ or $=N^+(R^7R^7)$ when X is $C(R^*R^{**})$; and/or

Z is a group of the formula: $-Z^*-Z^1-$ (linkage to L1, L2 or FETL), where:

Z^* is methylene, methoxy, ethoxy, aminomethyl, aminoethyl, aminopropynyl, aminobutynyl, carboxyethenyl, carboxyethynyl, optionally substituted aryl or optionally substituted heteroaryl,

Z^1 is $-C(O)-$, $-N(Z^2)-$, $-CH_2-O-$, $-CH_2-C(O)-$, $-CH_2-N(Z^2)-$, $-CH_2-S-$, $-CH_2-S(O)-$, $-CH_2-S(O_2)-$ or is absent, and

Z^2 is H, or is C₁ to C₈ lower alkyl or aryl optionally substituted with SO_3^- , COOH, NH₂, CH₂NH₂, SH, or SCH₃.

12. (Currently Amended) A fluorescence energy transfer dye represented by Formula II:

Probe--L1—(D1 or D2)—FETL-(low affinity false target) (Formula II)

where:

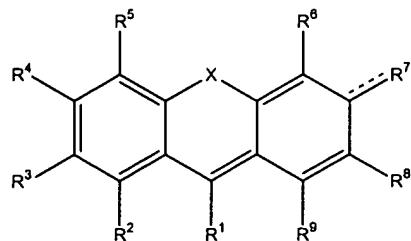
Probe is a polynucleotide, antibody, triglyceride, low density lipoprotein or lectin;

L1 is a link for attachment to a-the Probe;

FETL is a fluorescence energy transfer linker comprising a symmetric, rigid or sterically hindered, divalent moiety joined to D1 and D2 via an amine, carbonyl, activated carboxylic acid ester, disulfide, thiol or thiol ester;

low affinity false target is an analyte for the Probe disposed on FETL to deactivate, block or otherwise prevent coupling of FETL to a corresponding D2 or D1 until said low affinity false target is displaced by a higher affinity true target;

D1 is a donor dye represented by the formula:



D1

where:

X is O or $C(R^*R^{**})$, where R^* and R^{**} are independently lower alkyl or $-CH_2-Z$;

R^1 is H, CF₃, perfluoropropyl, lower alkyl acid, substituted aryl, substituted heteroaryl or Z;

R^2 is H, halo, SO_3^- , or is taken together with R^3 to form an optionally substituted fused ring having 5 to 7 atoms;

R^3 is halo, Z, or is taken together with R^2 and/or R^4 to form an optionally substituted fused ring having 5 to 7 atoms;

R^4 is =O or OH, $-N(R^4'R^{4''})$ or $=N^+(R^4'R^{4''})$, or is taken together with R^3 and/or R^5 to form an optionally substituted fused ring having 5 to 7 atoms,

where R^4' is H, lower alkyl or L1, and

$R^{4''}$ is H, lower alkyl or CH_2-Z ;

R^5 is H, halo, Z, or is taken together with R^4 to form an optionally substituted fused ring having 5 to 7 atoms;

R^6 is H, halo, Z, or is taken together with R^7 to form an optionally substituted fused ring having 5 to 7 atoms;

R^7 is =O or OH, $-N(R^7'R^{7''})$ or $=N^+(R^7'R^{7''})$, or is taken together with R^6 and/or R^8 to form an optionally substituted fused ring having 5 to 7 atoms,

where R^7' is H, lower alkyl or L1, and

$R^{7''}$ is H, lower alkyl or CH_2-Z ;

R^8 is halo, Z, or is taken together with R^7 and/or R^9 to form an optionally substituted fused ring having 5 to 7 atoms;

R^9 is H, halo, SO_3^- , or is taken together with R^8 to form an optionally substituted fused ring having 5 to 7 atoms; and

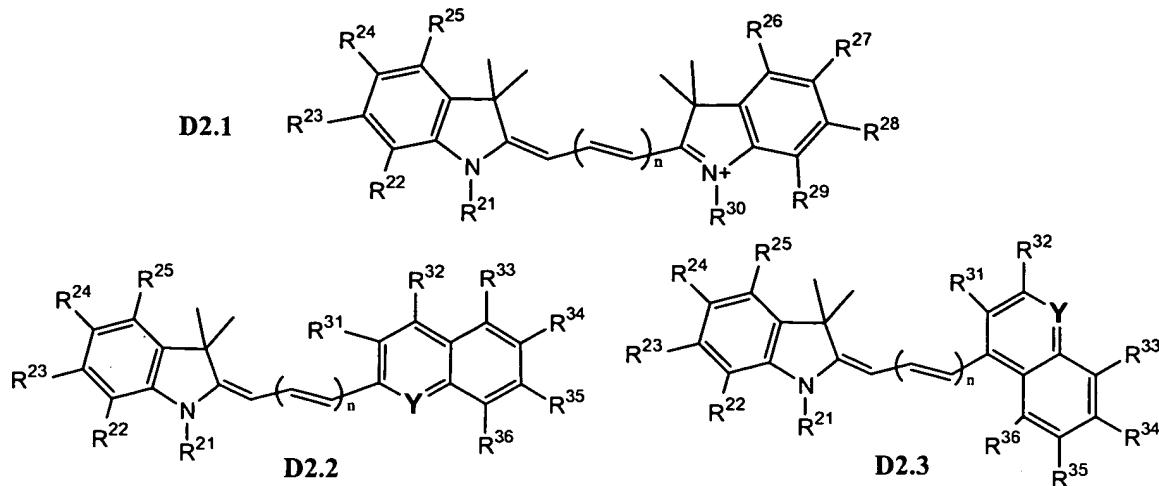
Z is a group of the formula: $-Z^*-Z^1-$ (linkage to L1, L2 or FETL), where:

Z^* is methylene, methoxy, ethoxy, aminomethyl, aminoethyl, aminopropynyl, aminobutynyl, carboxyethenyl, carboxyethynyl, optionally substituted aryl or optionally substituted heteroaryl;

Z^1 is $-C(O)-$, $-N(Z^2)-$, $-CH_2-O-$, $-CH_2-C(O)-$, $-CH_2-N(Z^2)-$, $-CH_2-S-$, $-CH_2-S(O)-$, $-CH_2-S(O_2)-$ or is absent; and

Z^2 is H, C_1 to C_8 optionally substituted lower alkyl, or optionally substituted aryl; and

D2 is an acceptor/reporter dye represented by formula D1 or by a formula of the group:



where:

at least one of R²¹ to R³⁶ is joined to FETL,

n is zero, 1, 2 or 3;

R²¹ and R³⁰ are independently -CH₂-Z, activated lower alkyl, or optionally substituted aryl; R²² to R²⁹ are independently H, SO₃⁻, or optionally substituted alkyl, or R²² and R²³, R²³ and R²⁴, R²⁴ and R²⁵, R²⁶ and R²⁷, R²⁷ and R²⁸, and/or R²⁸ and R²⁹ taken together form an optionally substituted fused ring having 6 atoms; and

R³¹ and R³² are independently H, optionally substituted alkyl, aryl, or taken together form an optionally substituted fused ring having 6 atoms;

R³³ to R³⁶ are independently H, SO₃⁻, optionally substituted alkyl, aryl, or R³³ and R³⁴, R³⁴ and R³⁵, and/or R³⁵ and R³⁶ taken together form an optionally substituted fused ring having 6 atoms; and

Y is -O- or -N(Y¹)- where Y¹ is -CH₂-Z, activated lower alkyl, or optionally substituted aryl or alkyl.

13. (Currently Amended) An A fluorescence energy transfer assay for determining the presence of a target site in a substance requiring no pre-analysis purification for removal of unbound dye, comprising the steps of:

- (a) contacting a substance to be tested and a target site specific Probe-L1-(D1 or D2)-FETL-(low affinity false target) conjugate of Claim 12 in a suitable assay vessel under conditions suitable for preferential binding of the Probe to the target site, as opposed to the low affinity false target;
- (b) introducing a second D2 or D1 fluorescence energy transfer dye having an activated site for coupling to the FETL into the vessel, under conditions suitable for coupling to FETL where the low affinity false target is not bound to the Probe, wherein the second dye is the other of the D1 or D2 found in the conjugate;

- (c) causing D1 to absorb energy; and
- (d) measuring the level of D2 emission, wherein emission from D2 upon absorption of energy by D1 indicates the presence of the target site in the substance.

14-16. (Cancelled)

17. (Currently Amended) A proximity assay comprising the steps of :

- (a) contacting a substance to be tested and a target-site-specific donor dye in a suitable assay vessel;
- (b) introducing a target-site-specific fluorescence energy transfer reporter dye of Claim 1 into the vessel, where said reporter dye's target is either spatially proximate to said donor dye target or specific for a given target to be tested for spatial proximity to said donor dye target, and said reporter dye's energy absorption spectra overlaps the emission spectra of said donor dye;
- (c) causing the donor dye to absorb energy; and
- (d) measuring the level of reporter dye emission, wherein emission from the reporter dye upon absorption of energy by the donor dye indicates the proximity of the donor dye target and the reporter dye target in the substance.

18. (Currently Amended) The proximity assay of Claim 17 wherein:

- step (b) further comprises introducing two or more target-site-specific fluorescence energy transfer reporter dye of Claim 1 into the vessel, said reporter dyes having energy absorption spectra overlapping the emission spectra of said donor dye, distinct emission spectra, and having different targets to be tested for spatial proximity to said donor dye target; and
- step (d) further comprises measuring the level of reporter dye emission at the wavelengths characteristic of said reporter dyes, wherein emission characteristic of each reporter dye upon absorption of energy by the donor dye indicates the respective proximity of the donor dye target to each corresponding reporter dye target in the substance.